FINAL REPORT
South Carolina State Wildlife Grants Project
South Carolina Department of Natural Resources
Evaluation of American shad stocking contribution in the Edisto River
T-58-R-1 F12AF01378
October 1, 2012 – September 30, 2015

Final Summary: Based on our proposed project timeline, tasks that were scheduled to be completed during the first two years of our project (October 1, 2012 - September 30, 2014) included (1) determining the contribution of the 2009 and 2010 year-class (YC) stockings of larval American shad to the 2012 and 2013 return spawning migrations in the Edisto River and (2) evaluating the genetic diversity and effective population size of American shad in the Edisto River during the 2012 and 2013 return spawning migrations to identify any potential influences from the 2009 and 2010 YC stockings on the natural population. Tasks that were scheduled to be completed during the third year of our project (October 1, 2014 - September 30, 2015) included (3) archiving ~100 broodstock fin clips from the 2014 production year and archiving ~300 2014 fall-collected juvenile samples, (4) processing ~200 American shad fin clips collected during the 2014 spawning migration in the Edisto River, (5) determining the contribution of all prior larval stockings (2009-2012 YC) of American shad to the 2014 return spawning migration in the Edisto River, and (6) evaluating the genetic diversity and effective population size of American shad in the Edisto River during the 2014 return spawning migration to identify any potential influences from prior stockings (2009-2012 YC) on the natural population. For this report, tasks with similar objectives were combined.

Sample collections (2012-2014): A total of 118 American shad were used as broodstock at Bear's Bluff National Fish Hatchery (BBNFH) in 2012, 74 shad were used in a 2012 telemetry study conducted by SCDNR, 17 shad were caught as field samples by BBNFH in 2012, and 96 shad were caught as field samples by commercial anglers in 2012. Seven samples were removed from these collections either because they did not amplify, were too contaminated to score, or were recaptures of the same individual. Therefore, a final total of 298 adult American shad samples collected in 2012 were genotyped and included in the data analysis.

A total of 75 American shad were used as broodstock at BBNFH in 2013, 21 shad were caught as field samples by BBNFH in 2013, and 95 shad were caught as field samples by commercial anglers in 2013. Eight samples were removed from these collections either because they did not amplify, were too contaminated to score, or were recaptures of the same individual. Therefore, a final total of 183 adult American shad samples collected in 2013 were genotyped and included in the data analysis.

A total of 44 American shad were used as broodstock at BBNFH in 2014, and 85 shad were caught as field samples by commercial anglers in 2014. Six samples were removed from these collections because they were too contaminated to score. Therefore, a final total of 123 adult American shad samples collected in 2014 were genotyped and included in the data analysis.

Genetic processing (2012-2014): All fin-clip samples were stored in a sarcosyl-urea solution (1% sarcosyl, 8M urea, 20mM sodium phosphate, 1mM EDTA). Genomic DNA was extracted using a metal bead isolation procedure. PCR amplifications were performed using a set of thirteen *Alosa*-specific microsatellite primers developed by Julian and Bartron (2007) for American shad. Markers were combined into three multiplexed PCR reactions containing dH2O,

1X HotMaster PCR Buffer (5 Prime Inc.; Gaithersburg, MD), 3.75mM $MgCl_2$, 0.16mg/ml BSA, 1.28mM dNTPs (0.32mM each), 0.71µM of forward and reverse primers, 0.05U/µl of HotMaster Taq (5 Prime Inc.), and 1µl of DNA (10-50ng/µl) for a total reaction volume of 15µl. PCR reactions were performed on BIORAD iCyclers (Bio-Rad Laboratories; Hercules, CA) using an initial denaturation step of 2 min at 94ºC, followed by 35 cycles of 94ºC for 45 sec, 60ºC for 45 sec, and 64ºC for 2 min, and ending with a final extension at 64ºC for 10 min. Amplified fragments were separated by capillary electrophoresis on a Beckman CEQ 8000 sequencing platform (Beckman Coulter; Brea, CA) using a 400bp size standard. Chromatograms were scored using CEQ™ Fragment Analysis Software (Beckman Coulter) with a frag 3/PA version 1 analysis algorithm to determine allele size. Two independent readers scored each chromatogram for quality assurance.

Tasks 1, 5: Determine the contribution of the 2009 and 2010 YC stockings of larval American shad to the 2012 and 2013 return spawning migrations in the Edisto River. Determine the contribution of all prior larval stockings (2009-2012 YC) of American shad to the 2014 return spawning migration in the Edisto River.

Accomplishments: To determine whether hatchery individuals from the 2009 and 2010 YC larval stockings contributed to the 2012 and 2013 return spawning migrations and whether hatchery individuals from the 2009-2012 YC larval stockings contributed to the 2014 return spawning migration, adult genotypes from the 2012 and 2013 collections were compared to the 2009 and 2010 broodstock and adult genotypes from the 2014 collections were compared to the 2009-2012 broodstock using CERVUS parentage analysis software (Kalinowski et al. 2007). Simulations (n = 5) for "sexes unknown" parentage analysis in CERVUS consisted of 10,000 offspring and 100 candidate parents (100% sampled) using allele frequencies generated from all samples of adult American shad. Critical delta values were determined using 99% confidence for the relaxed criteria and 100% for the strict. All parentage analyses were run with the modal simulation file. The percentage of hatchery contribution is determined as C/(W+C) x 100 where C is the number of cultured individuals and W is the number of wild individuals as designated by CERVUS at the strict confidence level, as no additional offspring were identified with the relaxed criteria.

No cultured fish from previous releases (2009 or 2010) were detected within the 2012 data set, indicating that hatchery contribution of the 2009 and 2010 YCs to the 2012 spawning migration in the Edisto River was 0%. No cultured fish from the 2009 release were detected within the 2013 data set, indicating that hatchery contribution of the 2009 YC to the 2013 spawning migration in the Edisto River was also 0%. One cultured fish from the 2010 release was detected within the 2013 data set, resulting in a hatchery contribution of 0.6% from the 2010 YC to the 2013 American shad spawning migration in the Edisto River. No cultured fish from previous releases (2009-2012) were detected within the 2014 data set, indicating that hatchery contribution of the 2009-2012 YCs to the 2014 spawning migration in the Edisto River was 0%.

The recapture of the 2010 YC fish in 2013 is the first documentation of a hatchery individual from the larval stockings of American shad in the Edisto River (2009-2014) returning during the adult spawning migration.

Significant deviations: There were no significant deviations for this objective.

<u>Tasks 2, 6:</u> Evaluate the genetic diversity and effective population size of American shad in the Edisto River during the 2012 and 2013 return spawning migrations to identify any potential influences from the 2009 and 2010 YC stockings on the natural population. Evaluate the genetic diversity and effective population size of American shad in the Edisto River during the 2014 return spawning migration to identify any potential influences from prior stockings (2009-2012 YC) on the natural population.

<u>Accomplishments</u>: To ensure the utility of our markers, tests for Hardy-Weinberg equilibrium (HWE), linkage disequilibrium, and null alleles were performed for all loci with the 2012, 2013, and 2014 Edisto River data sets. Examinations for HWE were conducted using exact tests performed with Markov Chain randomization in the program ARLEQUIN 3.5.1.3 (Excoffier et al. 2005). Chains had 1,000,000 steps with a 100,000 step burn-in. Tests for linkage equilibrium between all microsatellite pairs were executed in ARLEQUIN using 10,000 permutations. The frequency of possible null alleles at each locus was estimated in GENEPOP 4.2 (Rousset 2008). Significance levels for all analyses were adjusted using a sequential Bonferroni correction (Rice 1989). After correction for multiple comparisons, all loci in all years adhered to HWE and none of the inter-locus comparisons were significant. The probability of null alleles was relatively low (null frequency <0.05) for all loci in all years (Table 1). Therefore, the selected marker suite is appropriate for and has sufficient power to address the questions of the current study.

Basic molecular diversity indices including the number of alleles per locus, allelic size range, observed heterozygosity, expected heterozygosity, and inbreeding coefficients (F_{IS}; Weir and Cockerham 1984) were calculated for each locus and all loci together using ARLEQUIN and GENEPOP 4.2. FSTAT 2.9.3.2 (Goudet 1995, 2001) was used to estimate per-locus allelic richness. In 2012-2014, observed heterozygosity was high, both for individual loci (Table 1) and all loci taken together (2012 $H_O = 0.841$; 2013 $H_O = 0.855$; 2014 $H_O = 0.851$) with high levels of polymorphism (11-32 alleles per locus). Expected heterozygosity was also high for individual loci (Table 1) and all loci taken together (2012 $H_E = 0.848$; 2013 $H_E = 0.861$; 2014 $H_E = 0.858$). Allelic richness within collection years ranged from 11.0 to 32.0, and levels of inbreeding were low ($F_{IS} < 0.1$) for all loci individually (Table 1) and at the population level (2012 $F_{IS} = 0.008$; 2013 $F_{IS} = 0.007$; 2014 $F_{IS} = 0.008$).

Effective population size (N_e) estimates for the Edisto River 2012-2014 data sets were calculated using the program LDNe 1.2 (Waples and Do 2008). Genetic drift generates non-random associations among unlinked loci; LDNe analyzes this linkage disequilibrium between a set of loci to determine contemporary N_e for a single time point, producing several values based on allele frequency exclusion criteria. Waples and Do (2010) recommend excluding alleles at frequencies < 0.01 with sample sizes of 100 or more individuals (and at frequencies < 0.02 for sample sizes greater than 25 individuals). The correction for finite sampling originally developed by Waples (2006) often results in negative estimates in cases where the observed correlation of allele frequencies is low. Therefore, we report effective population size estimates using the lowest appropriate allele frequency exclusion criterion (based on sample size) that resulted in non-negative estimates. LDNe calculated an N_e estimate of 9,124 (2,689 - ∞) for 2012, 6,167 (1,730 - ∞) for 2013, and 3,771 (1,095 - ∞) for 2014. From the perspective of management and conservation, genetic effective size estimates for the Edisto River are substantially above the minimum value of 50 recommended to prevent significant inbreeding and maintain short-term fitness of a population (Franklin 1980) and exceed the minimum numbers suggested to maintain the evolutionary potential and long-term viability of a population (N_e = 500-1,000; Franklin and Frankham 1998, or N_e = 1,000-5,000; Lynch and Lande 1998). LDNe estimations of N_e in species with overlapping generations (such as American shad) may be downwardly biased as compared to the true population N_e (Waples et al. 2014). However, LDNe estimates are expected to approximate N_e when the number of

sampled cohorts is similar to the generation time of the organism (Waples and Do 2010; Waples et al. 2014). The Edisto River American shad spawning run generally consists of 4-6 cohorts (ages 2-8; see Figure 14.7 in ASMFC 2007b), which is similar to the generation time of American shad (4-5 years; Leggett and Carscadden 1978), so LDNe estimates should approximate N_e for the 2012-2014 Edisto River data sets.

Seven American shad data sets from the Edisto River, 2008 (n=31), 2009 (n=104), 2010 (n=363), 2011 (n=187), 2012 (n=298), 2013 (n=183), and 2014 (n=123), were compared to one another to determine whether there was a significant difference in allele frequencies between sampling years. A pair-wise comparison for temporal genetic variation was performed in ARLEQUIN. No significant temporal genetic differentiation ($p < 0.002$) was found between any of the collections (Table 2), providing evidence for a genetically stable spawning pool in the Edisto River over short time frames (7 years).

In summary, the genetic diversity of the American shad population in the Edisto River was high, effectively no inbreeding was detected, and effective population size estimates were on the order of several thousand individuals and within the levels recommended to maintain long-term fitness of the population. The spawning stock appears genetically stable as no significant temporal changes occurred between any of the collection years. These results are similar to those obtained for the pre-stocking collection years (2008-2011; Table 3).

Significant deviations: There were no significant deviations for this objective.

Task 3: Archive ~100 broodstock fin clips for the 2014 YC production year, and archive ~300 2014 fall-collected juvenile samples.

Accomplishments: Due to time constraints during the 2014 sampling season, only 44 American shad were collected for broodstock by BBNFH (see the "Sample collections" section above), rather than the ~100 that were anticipated, and no juvenile samples were collected in 2014. All of the broodstock that we received were archived in the SCDNR Genetic Tissue Collection database.

Significant deviations: There were no significant deviations for this objective.

Task 4: Process ~200 American shad fin clips collected during the 2014 spawning migration in the Edisto River.

Accomplishments: The total number of adult American shad collected in the Edisto River in 2014 was lower than in previous years (see the "Sample collections" section above), mainly due to the smaller number of broodstock collected for the 2014 hatchery production. A total of 129 samples were collected during the 2014 spawning migration; all of which were genotyped using the methods described above in the "Genetic processing" section.

Significant deviations: There were no significant deviations for this objective.

Overall Project Conclusions: A reduction in catch-per-unit-effort for American shad in the Edisto River over the past few decades highlighted the need to obtain river-specific information for this system and assess the potential of responsible stocking as an effective management tool. Through a collaborative effort among SCDNR's Wildlife and Freshwater Fisheries Division, SCDNR's Marine Resources Division, and the USFWS (Bear's Bluff National Fish Hatchery), six year classes of American shad larvae (2009-2014) have been produced/stocked and genetic markers have been optimized to allow for estimates of hatchery contribution using genetic parentage analysis. Our initial genetic evaluation on juvenile samples collected in 2008-2011 found that even with low stocking levels (< 22,000 larvae) hatchery fry could be detected among the out-migrating juvenile American shad (up to 3.5%). Our initial evaluation of adult samples collected in 2008-2011 also generated critical baseline genetic data for the Edisto River American shad spawning run, indicating the genetic composition of the Edisto spawning run is temporally stable over short time frames, genetic diversity is high and inbreeding is low, and effective population size is relatively large and within the levels recommended to maintain the population's evolutionary potential.

Our further investigations included adult samples collected from 2012-2014. We wanted to evaluate the effectiveness of the stock enhancement efforts by determining whether hatchery fish recruit into the adult reproductive pool (i.e., during their return spawning migration). The spawning runs of 2012-2014 represented the first opportunities for the 2009YC-2012 YC hatchery fish to return to the Edisto River as reproductive adults. A single hatchery individual from the 2010YC was detected during the 2013 spawning run. We now have documented evidence that stocked larval American shad in the Edisto River can survive to adulthood and return to reproduce, thus contributing to the Edisto River American shad population. Although hatchery contribution is low (< 1%), this level is not unexpected given the numbers of fry released (2,465-22,209). Full-scale stockings of American shad in U.S. Atlantic coastal rivers often include the release of anywhere from approximately 100,000 to over 10 million fry and hatchery contributions of 30% or more have been reported (ASMFC 2007a,b). Considering the number of fry stocked during our trial project, a ~1% hatchery contribution is a successful outcome for our experimental-level releases. In summary, the detection of hatchery individuals within the wild stock suggests that stock enhancement does have the potential to be a viable fisheries management tool for American shad restoration in the Edisto River.

We also wanted to identify any potential influences of hatchery fish on the genetic health of the wild Edisto River American shad population (e.g. reduction of genetic diversity, increase in inbreeding, decrease in effective population size, significant temporal differences between collection years). We compared the 2008-2011 collection years, which represent the genetic composition of the Edisto River before stocking, to the 2012-2014 collection years, which represent the genetic composition of the Edisto River after stocking. We found that genetic diversity and inbreeding measures did not change appreciably between collection years (Table 3). Furthermore, there was no pattern of decline in effective population size between pre- and post-enhancement periods (Table 3), and there was no significant genetic differentiation between collection years which might indicate changes in allele frequencies (Table 2). Therefore, we do not see any evidence that our experimental-scale stocking has had any unintended impacts on the genetic composition of the Edisto River American shad spawning run, although population genetic parameters should continue to be monitored as other year-classes return to spawn.

Estimated Federal Cost: $94,295

Recommendations: Close the grant.

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Tables:

Table 1. Number of alleles, allelic size range, allelic richness (R), observed heterozygosity (H_O), expected heterozygosity (H_E), F_{IS} values, p-values for Hardy-Weinberg equilibrium tests (HWE), and the frequency of potential null alleles for each locus in the 2012-2014 Edisto River data sets.

Collection Year	Locus Name	Alleles	Range	R	H_O	H_E	F_{IS}	HWE	Null
2012	AsaD030	24	104-204	24.0	0.950	0.925	-0.027	0.967	0.000
	AsaD031	14	182-242	14.0	0.826	0.863	0.043	0.416	0.023
	AsaC010	22	249-345	22.0	0.886	0.902	0.018	0.158	0.007
	AsaD429	11	135-175	11.0	0.791	0.790	-0.001	0.183	0.014
	AsaD021	14	251-303	14.0	0.889	0.865	-0.028	0.593	0.002
	AsaD312	17	128-204	17.0	0.879	0.885	0.006	0.930	0.001
	AsaC059	15	279-359	15.0	0.792	0.819	0.033	0.190	0.014
	AsaB020	12	116-152	12.0	0.705	0.713	0.012	0.652	0.007
	AsaD055	15	227-283	15.0	0.804	0.797	-0.009	0.774	0.000
	AsaC334	30	235-355	30.0	0.896	0.903	0.008	0.613	0.000
	AsaC249	27	104-182	27.0	0.851	0.861	0.011	0.160	0.013
	AsaC051	18	250-318	18.0	0.782	0.796	0.018	0.172	0.000
	AsaD042	18	148-216	18.0	0.883	0.907	0.027	0.715	0.011
2013	AsaD030	24	104-200	24.0	0.862	0.922	0.066	0.215	0.034
	AsaD031	13	182-238	13.0	0.814	0.870	0.065	0.147	0.034
	AsaC010	21	261-349	21.0	0.923	0.902	-0.023	0.004	0.000
	AsaD429	11	135-175	11.0	0.858	0.836	-0.027	0.458	0.007
	AsaD021	14	251-303	14.0	0.884	0.856	-0.033	0.846	0.000
	AsaD312	17	124-192	17.0	0.902	0.888	-0.016	0.086	0.000
	AsaC059	17	267-359	17.0	0.809	0.853	0.053	0.235	0.022
	AsaB020	12	116-149	12.0	0.760	0.725	-0.048	0.584	0.007
	AsaD055	12	227-275	12.0	0.787	0.807	0.026	0.776	0.024
	AsaC334	32	235-367	32.0	0.896	0.910	0.016	0.028	0.008
	AsaC249	27	104-182	26.9	0.874	0.900	0.029	0.231	0.006
	AsaC051	17	242-314	17.0	0.820	0.811	-0.010	0.469	0.000
	AsaD042	19	148-232	19.0	0.951	0.903	-0.053	0.078	0.000
2014	AsaD030	23	104-200	23.0	0.935	0.925	-0.010	0.642	0.000
	AsaD031	13	182-242	13.0	0.878	0.871	-0.009	0.962	0.000
	AsaC010	18	261-337	18.0	0.894	0.912	0.019	0.082	0.009
	AsaD429	12	135-179	12.0	0.846	0.816	-0.037	0.770	0.000
	AsaD021	13	251-299	13.0	0.837	0.849	0.014	0.762	0.000
	AsaD312	16	128-188	16.0	0.883	0.895	0.013	0.987	0.008
	AsaC059	16	271-363	15.9	0.797	0.825	0.035	0.025	0.000
	AsaB020	11	116-149	11.0	0.724	0.755	0.041	0.331	0.022
	AsaD055	13	227-279	12.9	0.829	0.822	-0.009	0.940	0.000
	AsaC334	23	104-184	23.0	0.875	0.877	0.002	0.972	0.009
	AsaC249	28	235-355	28.0	0.878	0.919	0.044	0.610	0.016
	AsaC051	15	250-314	15.0	0.764	0.781	0.021	0.827	0.018
	AsaD042	19	148-224	19.0	0.926	0.908	-0.020	0.307	0.000

Table 2. F_{ST} estimates (below diagonal) and corresponding p-values (above diagonal) for pair-wise comparisons between the Edisto River 2008-2014 adult American shad data sets. Critical p value is ≤0.002 for statistical significance; no comparisons are significant.

Data Set	2008	2009	2010	2011	2012	2013	2014
2008	X	0.281	0.604	0.188	0.350	0.681	0.455
2009	0.00094	X	0.127	0.555	0.026	0.043	0.069
2010	-0.00033	0.00056	X	0.590	0.259	0.390	0.703
2011	0.00121	-0.00011	-0.00008	X	0.863	0.422	0.637
2012	0.00049	0.00102	0.00015	-0.00034	X	0.042	0.655
2013	-0.00064	0.00105	0.00007	0.00004	0.00060	X	0.847
2014	0.00012	0.00105	-0.00021	-0.00022	-0.00017	-0.00049	X

Table 3. A comparison of the average allelic richness (R), observed heterozygosity (H_O), expected heterozygosity (H_E), F_{IS} values, and effective population size among the 2008-2011 collection years (pre-stocking) and the 2012-2014 collection years (post-stocking) of American shad in the Edisto River. Allelic richness estimates are based on a sample of 28 diploid individuals for comparison across collection years. A numerical estimate of effective size could not be calculated for the 2010 collection.

Year	Sample Size	Average R	Average H_O	Average H_E	Average F_{IS}	Effective Size
2008	31	12.3	0.822	0.856	0.040	3,505 (243 - ∞)
2009	104	11.9	0.843	0.850	0.009	8,379 (883 - ∞)
2010	363	12.3	0.845	0.856	0.013	large
2011	187	12.1	0.858	0.853	-0.006	2,019 (1,067 - ∞)
2012	298	12.1	0.841	0.848	0.008	9,124 (2,689 - ∞)
2013	183	12.6	0.855	0.861	0.007	6,167 (1,730 - ∞)
2014	123	12.4	0.851	0.858	0.008	3,771 (1,095 - ∞)